Development of Recombinant Reader Proteins for Epigenetic Profiling in Genomic Assays

Anup Vaidya[1], Allison R. Hickman[1], Laiba Khan[1], Nathaniel Tate Burkholder[2], Jake Foreman[2], Matthew R. Marunde[1], Saarang Gopinath[1], Matthew J. Meiners[1], Jonathan M. Burg[1], Zu-Wen Sun[1], Martis W. Cowles[1], Brian D. Strahl[2] & Michael-Christopher Keogh[1]

[1] EpiCypher Inc, Durham, NC 27709
[2] University of North Carolina, Chapel Hill, NC 27599

Challenges in Using Antibodies as Genomic Mapping Tools

Efficient packaging of DNA within the eukaryotic cell nucleus is achieved by wrapping DNA around an octamer of core histone proteins (H2A, H2B, H3 and H4) that are subject to extensive post translational modifications (PTMs). These PTMs play critical roles regulating gene expression, DNA recombination, repair and cellular differentiation. Thus, their mechanistic dissection is of central importance in understanding development and disease **(1)**.

Reliable mapping of histone PTMs requires reagents that can demonstrate robust / specific binding towards the intended target, while showing minimal cross-reactivity to related entities. Traditional methods of histone PTM profiling have heavily relied on antibodies that suffer from poor efficiency and specificity **(2)**. Even when capable antibodies are identified, lot-to-lot variability can make it difficult to produce reproducible data. Further, there are several PTMs with disease relevance but no capable commercial antibodies. EpiCypher is partnering with the Strahl lab to develop recombinant reader proteins as effective tools for histone PTM profiling in genomic assays. We leverage our fully-defined nucleosomes to evaluate the specificity of epigenetic readers and then use spike-in barcoded nucleosomes to demonstrate that these proteins can effectively target histone PTMs in CUT&RUN. **Here, we describe use of the Bromo-PWWP domains from ZMYND11 (targeting H3.3K36me3) as a histone PTM mapping tool for genomic assays.**

Table 1: Comparison between antibodies and epigenetic reader proteins as genomic mapping tools

Antibodies	Reader Proteins
Variable Specificity	Variable Specificity
Variable Efficiency	Low Affinity (but paired domains can synergize for affinity or specificity)
High lot-to-lot Variation	Low lot-to-lot Variation
Difficult to Functionalize	Easy to Functionalize
Animal Lives Expended	Can be produced in *E.coli*

Histone Variant H3.3

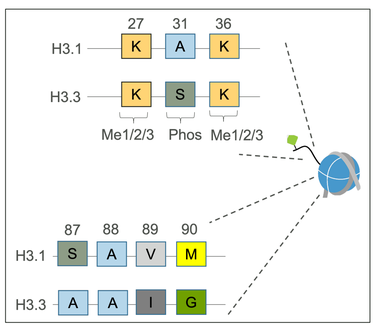


Figure 1: Sequence differences between H3.1 and H3.3. Variant H3.3 differs from the canonical H3.1 at residues 31 (the tail: A for H3.1 vs S for H3.3) and 87-90 (the core region). S31 is phosphorylated during stress-response while [87-90] specifies the association with particular histone chaperones. **For antibodies to differentiate H3.1 and H3.3**, A/S31 is available in an intact nucleosome, while the chaperone interface is the tetramer interface and thus sterically inaccessible.

H3.3 is a H3 variant that differs from canonical H3.1/H3.2 at positions 31 and 87-90 (**Figure 1**). In contrast to canonical H3 (cell-cycle regulated [S phase], CAF-chaperone associated, and replication-dependent deposition), H3.3 deposition is cell-cycle independent, HIRA-chaperone associated and replication-independent. A major region of H3.3 genomic enrichment is the gene bodies of actively transcribed genes. H3.3 is the target of several 'oncomutations' including K27M, G34W and K36M in pediatric cancers **(3)**. Therefore, studying H3.3-associated PTMs and their reader proteins can play a crucial role in identifying tumor regulators. One such tumor suppressor **ZMYND11** (**Figure 3**) binds H3.3K36me3 in the context of S31, with phosphorylation of this residue disrupting binding **(4,5)**.

Most H3K36me3 Antibodies do not Differentiate Between H3.1 and H3.3

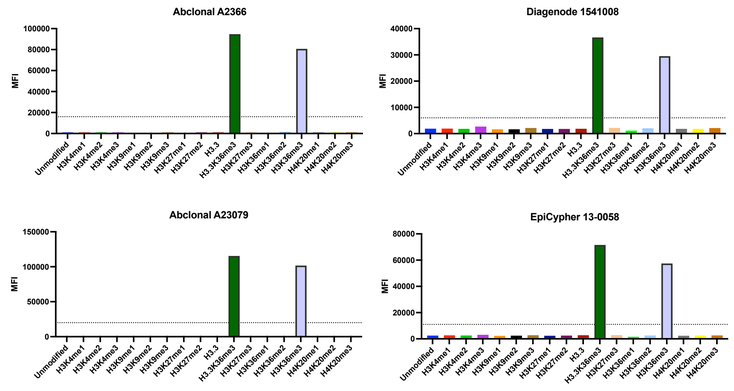


Figure 2: **Commercial H3K36me3 antibodies do not differentiate between H3.1 and H3.3.** Luminex counts for different H3K36me3 antibodies evaluated against a panel of fully defined nucleosomes. The tested antibodies tested failed to distinguish between H3.1 (A31) and variant H3.3 (S31). Dotted line indicates 20% of target (H3.1K36me3) counts.

ZMYND11 as a Model to Demonstrate Utility of Reader CUT&RUN

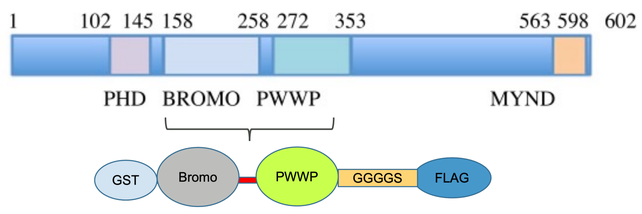


Figure 3: Schematic of BS69/ZMYND11 structure. ZMYND11 contains multiple domains with reader potential. The [Bromo / PWWP **(BP)**] domains have been shown to bind H3.3K36me3 with higher affinity than H3.1K36me3 **(5)**. The construct used for our study encompasses BP (residues 154-371), with N-terminal GST and C-terminal FLAG tags.

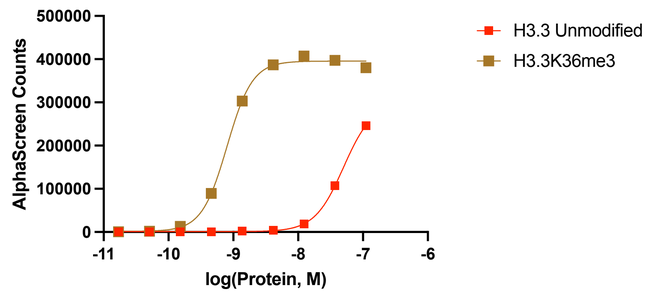


Figure 4. Alpha characterization of GST tagged ZMYND11-BP. *EpiCypher* **dCypher v2.0** uses **no-wash** Alpha technology to examine the binding of epitope tagged queries (*e.g.,* GST tagged ZMYND11-BP) and biotinylated targets (*e.g.,* fully-defined nucleosomes) **in parallel-wells**. GST-ZMYND11-BP shows a strong preference for nucleosomes bearing H3.3K36me3 over unmodified H3.3.

ZMYND11 (also known as BS69) was originally identified as a transcriptional regulator involved in pre mRNA processing, and its dysregulation has been associated with several cancers. Its Bromo and PWWP domains are critical for recognizing H3.3K36me3. ZMYND11 is predominantly enriched in the gene bodies of actively transcribed genes and is postulated to be recruited there by elongating RNA polymerase II **(4,5)**. Our study utilized a GST tagged version of ZMYND11 containing the Bromo and PWWP domains (GST-ZMYND11-BP) to map H3.3K36me3 (**Figure 3**).

dCypher Can be Used to Evaluate Reader Binding

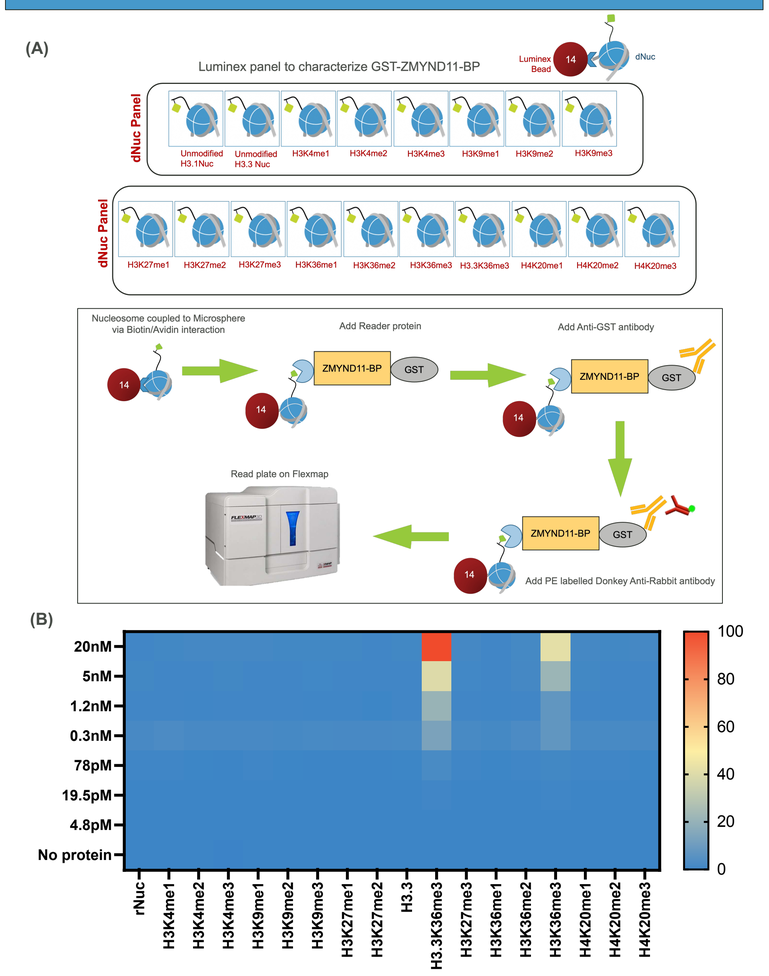


Figure 5. Luminex characterization of GST-ZMYND11-BP. (**A-B**) *EpiCypher* **dCypher v3.0** uses **wash-compatible** Luminex technology (**A**) to examine the binding of epitope-tagged queries (*e.g.,* GST-ZMYND11-BP) and biotinylated targets (*e.g.,* fully-defined nucleosomes) **in multiplex**. dCypher v3.0 can be used to optimize conditions for genomic assays like CUT&RUN. **(B)** GST-ZMYND11-BP shows preferential binding towards H3.3K36me3 over H3.1K36me3.

Reader CUT&RUN- Mapping Targets Using Recombinant Reader Proteins

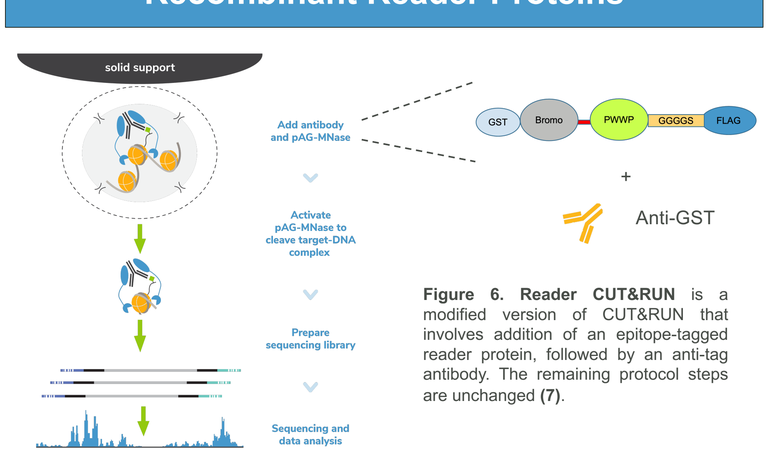
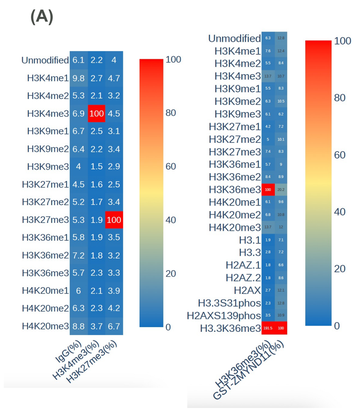


Figure 6. Reader CUT&RUN is a modified version of CUT&RUN that involves addition of an epitope-tagged reader protein, followed by an anti-tag antibody. The remaining protocol steps are unchanged **(7)**.

Highly Specific Genomic Profiling using Reader CUT&RUN

(A) *EpiCypher* spike in controls are invaluable to monitor specific enrichment in reader CUT&RUN



GST-ZMYND11-BP is enriched at gene locations harboring both H3.3 and H3K36me3 (B) and genes that are actively transcribed (C)

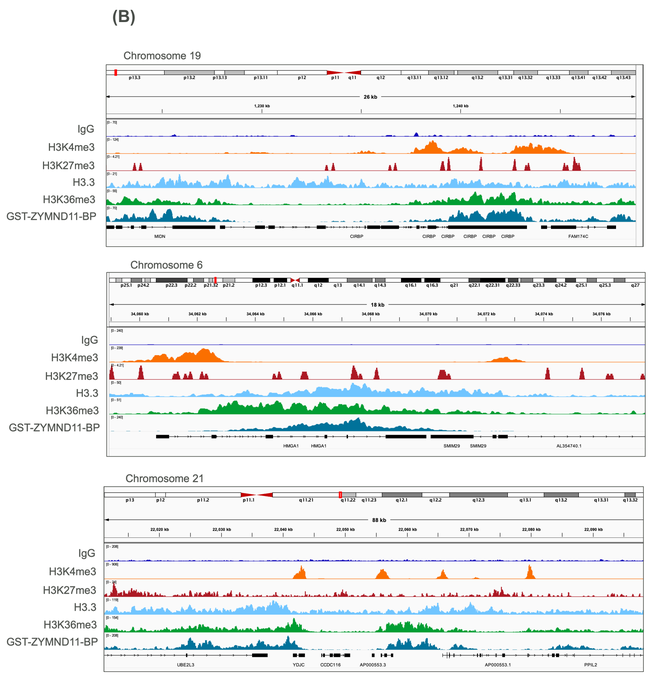


Figure 7. Reader CUT&RUN using GST-ZMYND11-BP. A) GST-ZMYND11-BP was used in a reader CUT&RUN experiment including biotinylated nucleosome spike-in controls. Note preferential enrichment of the H3.3K36me3 spike-in relative to other panel members. (**B**) Genome browser tracks show GST-ZMYND11-BP enrichment at regions harboring both H3.3 and H3K36me3 (Note: GST-ZMYND11-BP group scaled with IgG). **(C)** Comparison of CUT&RUN and K562 RNA-seq data reveals GST-ZMYND11-BP enrichment across protein-coding genes correlates with their RNA levels.

Conclusions

- Reader CUT&RUN is a powerful technology to map challenging PTMs for which commercial antibodies are currently lacking
- Reader CUT&RUN is amenable to the use of spike-in nucleosome panels to dissect / monitor binding and produce high quality data
- dCypher 3.0 binding trends can be used to inform specificity for future reader CUT&RUN experiments
- Reader CUT&RUN gives access to study co-occurring PTMs **(10)**

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